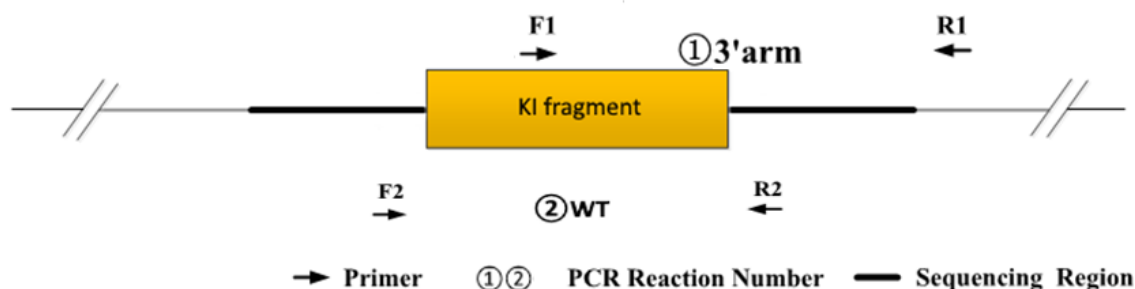


Genotyping Report

Strain ID	T053304	Strain Type	KI(Cas9)	Genetic Background	C57BL/6JGpt
Designer	Dongdong Zhang	Gene Name	Six2-tdTomato		

1. Strategy of Genotyping



Wild type: ①PCR reaction obtains none band; ②PCR reaction obtains a WT band.

Heterozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains a WT band.

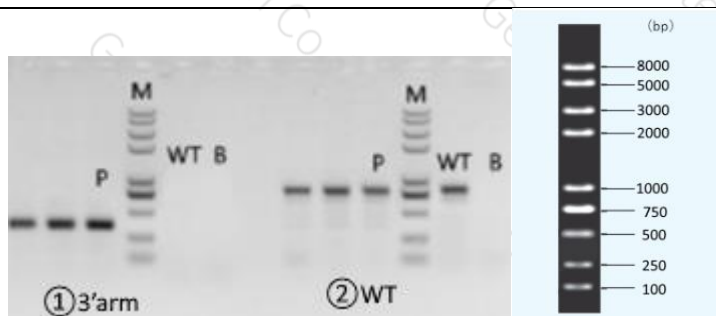
Homozygote: ①PCR reaction obtains a Targeted band; ②PCR reaction obtains none band.

Note: The sizes of WT and Targeted band are shown below. For ②PCR reaction, because the WT band is much smaller than the target band, it is likely to produce dominant amplification, the reaction is only used to judge whether there is a WT allele.

2. Primer Information

PCR No.	Primer No.	Sequence	Band Size
①3'arm	T053304-F1	GCATCGCATTGTCTGAGTAGGTG	WT:0bp Targeted:327bp
	T053304-R1	AGAACTGGTGGCTCTCCAGGATT	
②WT	T053304-F2	ACGGATCGTTGTGACTCAGGAGT	WT:806bp Targeted:5042bp
	T053304-R2	CGATTTAGTCCTGTCCAGGAGCT	

3. Gel Image & Conclusion



Note: P:Positive control; WT: Wildtype control; B: Blank control (ddH₂O); M: DNA Ladder

4. PCR Condition

PCR Reaction Component			
Seg.	reaction component		Volume (μl)
1	2 × Taq Master Mix , Dye Plus, (Vazyme P112-03)		12.5
2	ddH2O		9.5
3	Primer A(10pmol/μl)		1
4	Primer B(10pmol/μl)		1
5	Template(≈100ng/μl)		1
PCR program			
Seg.	Temp.	Time	Cycle
1	95°C	5min	20×
2	98°C	30s	
3	65°C (-0.5°C/cycle)	30s	
4	72°C	45s	
5	98°C	30s	20×
6	55°C	30s	
7	72°C	45s	
8	72°C	5min	
9	10°C	hold	